

Practitioner's Docket No. 49477 (1958)

514 Rec'd PCT/PTO/446996  
30 DEC 1999

CHAPTER II

TRANSMITTAL LETTER  
TO THE UNITED STATES ELECTED OFFICE (EO/US)  
(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/DE98/01902 3 July 1998 4 July 1997  
INTERNATIONAL APPLICATION NO. INTERNATIONAL FILING DATE PRIORITY DATE CLAIMED

METHOD FOR RECOGNIZING AND DETERMINING GNRH RECEPTORS AND THE USE OF  
GNRH AGONISTS AND GNRH ANTAGONISTS AND OTHER GNRH RECEPTOR LIGANDS  
FOR THE TREATMENT WITH GNRH RECEPTORS OF TUMORS ORIGINATING IN THE  
BRAIN AND/OR NERVOUS SYSTEM AND/OR MENINGES AND/OR OF KAPOSI SARCOMA  
TITLE OF INVENTION

Johannes Christianus VAN GROENINGHEN

APPLICANTS

Box PCT  
Assistant Commissioner for Patents  
Washington D.C. 20231  
ATTENTION: EO/US

**NOTE:** *To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.*

**WARNING:** *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. §1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 C.F.R. §1.8.*

**NOTE:** *Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 C.F.R. § 1.494(f).*

CERTIFICATION UNDER 37 C.F.R. § 1.10\*

(Express Mail label number is mandatory.)

(Express Mail certification is optional.)

I hereby certify that this paper, along with any document referred to, is being deposited with the United States Postal Service on this date December 30, 1999 in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL299775628US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Susan M. Dillon  
(type or print name of person mailing paper)

Susan M. Dillon  
Signature of person mailing paper

**WARNING:** *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

**\*WARNING:** *Each paper or fee filed by "Express Mail" must have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*

*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition."*  
Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

- This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
- The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

2. Fees

| CLAIMS FEE<br>[ ]*                                     | (1) FOR  | (2) NUMBER FILED | (3) NUMBER EXTRA | (4) RATE     | (5) CALCULATIONS |
|--|--|------------------|------------------|--------------|------------------|
|  | TOTAL CLAIMS   | 14 - 20 =        | 0                | x \$ 18.00 = | \$0              |
|  | INDEPENDENT CLAIMS   | 6 - 3 =          | 3                | x \$ 78.00 = | \$234.00         |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) + \$260.00 |  |                  |                  |              | \$260.00         |
| BASIC FEE**  | <input type="checkbox"/> U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY<br>Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO:<br><input type="checkbox"/> and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(2) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4)) ..... \$96.00<br><input type="checkbox"/> and the above requirements are not met (37 CFR 1.492(a)(1)) ..... \$670.00 |                  |                  |              |                  |
|  | <input checked="" type="checkbox"/> U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY<br>Where no international preliminary examination fee as set forth in § 1.482 has been paid to the USPTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO:<br><input type="checkbox"/> has been paid (37 CFR 1.492(a)(2)) ..... \$760.00<br><input type="checkbox"/> has not been paid (37 CFR 1.492(a)(3)) ..... \$970.00<br><input checked="" type="checkbox"/> where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) ..... \$840.00             |                  |                  |              |                  |
| Total of above Calculations                            |  |                  |                  |              | = \$1,334.00     |
| SMALL ENTITY   | Reduction by ½ for filing by small entity, if applicable. Affidavit must be filed. (note 37 CFR 1.9, 1.27, 1.28)   |                  |                  |              |                  |
|  | Subtotal   |                  |                  |              |                  |
|  | Total National Fee   |                  |                  |              |                  |
|  | Fee for recording the enclosed assignment document \$40.00 (37 CFR 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".   |                  |                  |              |                  |
| TOTAL  | Total Fees enclosed  |                  |                  |              | \$667.00         |

\*See attached Preliminary Amendment Reducing the Number of Claims.

09/446996  
514 Rec'd PCT/PTO 30 DEC 1999

i.  A check in the amount of \$667.00 to cover the above fees is enclosed.  
ii.  Please charge Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_.  
A duplicate copy of this sheet is enclosed.

**\*\*WARNING:** *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: \* \* \* (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

**WARNING:** *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3.  A copy of the International application as filed (35 U.S.C. 371(c)(2)):

**NOTE:** *Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.*

a.  is transmitted herewith.  
b.  is not required, as the application was filed with the United States Receiving Office.  
c.  has been transmitted  
i.  by the International Bureau.  
Date of mailing of the application (from form PCT/IB/308): \_\_\_\_\_  
ii.  by applicant on \_\_\_\_\_  
Date

4.  A translation of the International application into the English language (35 U.S.C. 371(c)(2)):

a.  is transmitted herewith.  
b.  is not required as the application was filed in English.  
c.  was previously transmitted by applicant on \_\_\_\_\_  
Date  
d.  will follow.

5.  Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

**NOTE:** *The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.*

a. [ ] are transmitted herewith.  
 b. [ ] have been transmitted  
 i. [X] by the International Bureau.  
 Date of mailing of the amendment (from form PCT/IB/308): \_\_\_\_\_  
 ii. [ ] by applicant on \_\_\_\_\_  
 Date  
 c. [X] have not been transmitted as  
 i. [X] applicant chose not to make amendments under PCT Article 19.  
 Date of mailing of Search Report (from form PCT/ISA/210): 09/02/99  
 ii. [ ] the time limit for the submission of amendments has not yet expired.  
 The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):  
 a. [ ] is transmitted herewith.  
 b. [ ] is not required as the amendments were made in the English language.  
 c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)  
 [X] is transmitted herewith.  
 [ ] is not required as the application was filed with the United States Receiving Office.

8. [X] Annex(es) to the international preliminary examination report  
 a. [X] is/are transmitted herewith.  
 b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [X] A translation of the annexes to the international preliminary examination report  
 a. [X] is transmitted herewith.  
 b. [ ] is not required as the annexes are in the English language.

10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115  
 a. [ ] was previously submitted by applicant on \_\_\_\_\_  
 Date  
 b. [X] is submitted herewith, and such oath or declaration  
 i. [X] is attached to the application.  
 ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.  
 iii. [ ] will follow.

Other document(s) or information included:

11. [X] An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):  
 a. [X] is transmitted herewith.  
 b. [ ] has been transmitted by the International Bureau.

c. [ ] Date of mailing (from form PCT/IB/308): \_\_\_\_\_  
is not required, as the application was searched by the United States  
International Searching Authority.

d. [ ] will be transmitted promptly upon request.

e. [ ] has been submitted by applicant on \_\_\_\_\_  
Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:  
a. [X] is transmitted herewith.  
Also transmitted herewith is/are:  
[X] Form PTO-1449 (PTO/SB/08A and 08B).  
[X] Copies of citations listed.

b. [ ] will be transmitted within THREE MONTHS of the date of submission of  
requirements under 35 U.S.C. 371(c).

c. [ ] was previously submitted by applicant on \_\_\_\_\_  
Date

13. [ ] An assignment document is transmitted herewith for recording.

A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING  
NEW PATENT APPLICATION" or [ ] FORM PTO  
1595 is also attached.

---

---

---

14. [X] Additional documents:  
a. [X] Copy of request (PCT/RO/101)  
b. [X] International Publication No. WO 99/00494  
i. [ ] Specification, claims and drawing  
ii. [X] Front page only  
c. [X] Preliminary amendment (37 C.F.R. § 1.121)  
d. [X] Other

Written Opinion

Response to Written Opinion

Form PCT/IB/306 (Change of Applicant's address)

15. [X] The above checked items are being transmitted  
a. [X] before 30 months from any claimed priority date.  
b. [ ] after 30 months.

16. [ ] Certain requirements under 35 U.S.C. 371 were previously submitted by the  
applicant on \_\_\_\_\_, namely:  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**AUTHORIZATION TO CHARGE ADDITIONAL FEES**

**WARNING:** *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

**NOTE:** *"A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

**NOTE:** *"Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).*

The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 04-1105.

37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

**WARNING:** *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)

**NOTE:** *Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.*

37 C.F.R. 1.17 (application processing fees)

37 C.F.R. 1.17(a)(1)-(5)(extension fees pursuant to § 1.136(a)).

37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

**NOTE:** *Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).*

**NOTE:** *37 C.F.R. 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.*

37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

Christi C. Oday  
SIGNATURE OF PRACTITIONER

**SIGNATURE OF PRACTITIONER**

Reg. No.: 38,256

Christine C. O'Day

*(type or print name of practitioner)*

Tel. No.: (617) 523-3400

Dike, Bronstein, Roberts & Cushman, LLP

130 Water Street

P.O. Address

Customer No.:

Boston, MA 02109

09/446996  
514 Rec'd PCT/PTO 30 DEC 1999  
Docket No. 49477

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT: Johannes Christianus VAN GROENINGHEN

SERIAL NO.: Not Yet Assigned  
[Express Mail Label No. EL299775628US]

FILED: Herewith

FOR: METHOD FOR RECOGNIZING AND DETERMINING GNRH  
RECEPTORS AND THE USE OF GNRH AGONISTS AND GNRH  
ANTAGONISTS AND OTHER GNRH RECEPTOR LIGANDS FOR THE  
TREATMENT WITH GNRH RECEPTORS OF TUMORS  
ORIGINATING IN THE BRAIN AND/OR NERVOUS SYSTEM  
AND/OR MENINGES AND/OR OF KAPOSI SARCOMA

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

**PRELIMINARY AMENDMENT**

Please amend the above-identified application as follows:

**IN THE SPECIFICATION**

Kindly replace page 9 of the specification with the substitute sheet submitted herewith.

**IN THE CLAIMS**

8. (amended) Diagnostic kit according to claim 7 for the detection of GnRH receptors for immunohistological diagnostics, for monitoring of the therapy, aftercare, early recognition of recidivation, and early recognition of tumors originating in brain and/or nervous system and/or the meninges comprising either a GnRH agonist or a monoclonal or polyclonal antibody against GnRH receptors, or one or more specific primer for GnRH receptors, e.g. for the amplification of GnRH receptor DNA in a reverse transcriptase polymerase chain reaction (RT-PCR).

APPLICANT: Johannes Christianus VAN GROENINGHEN  
SERIAL NO.: Not Yet Assigned  
[Express Mail Label No. EL299775628US]

Page 2

13. (amended) Use according to claim 10, whereby the [Conjugate of a] GnRH agonists or GnRH antagonists are used in combination with [to] a cytotoxic substance.

**REMARKS**

A substitute sheet for page 9 of the specification is submitted herewith merely to correct information appearing in Table 1 for the trademarks Antide® and Ramorelix®.

Further, claims 8 and 13 have been amended merely to reflect changes effected during the international phase of corresponding Application No. PCT/DE98/01902. No new matter has been added by virtue of these amendments.

Early consideration and allowance of the application are earnestly solicited.

Respectfully submitted,

*Christine C. O'Day*

---

Christine C. O'Day (Reg. 38,256)  
Peter F. Corless (Reg. 33,860)  
DIKE, BRONSTEIN, ROBERTS  
& CUSHMAN, LLP  
130 Water Street  
Boston, MA 02109-4280  
(617) 523-3400

#132530

Table I

List of GnRH agonists and GnRH antagonists which may be employed in the treatment of a tumor having GnRH receptors and originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma:

| <u>GnRH agonists:</u>           | <u>GnRH antagonists:</u>                             |
|---------------------------------|--|
| Pharmacological substance name  | Trade mark   |
| Leuprorelinacetate, Leuprorelin | Cetrorelix®, Asta Medica AG, Frankfurt/Main          |
| Triptorelinacetate, Triptorelin | Antarelix®, Asta Medica AG, Frankfurt/Main           |
| Buserelinacetate, Buserelin     | Antide®, Ares Sarono Int. SA, Genève,<br>Switzerland |
| Goserelinacetate, Goserelin     | Ramorelix®, Hoe013HoechstGmbH, Frankfurt/Main        |

Further examples of GnRH antagonists are:

- LHRH antagonists similar to Antide® as described in US patent 5,480,969  
(Bowers et al., date of patent: Jan. 2, 1996)
- LHRH peptide derivatives as described in the UK patent GB 2 246782 B  
(Albert, R., et al., patent published on 16/09/1992)
- LHRH antagonists as described in US patent 5,198,533  
(Schally et al., date of patent: Mar. 30, 1993)

The minimum treatment dose of the GnRH agonists in the above list corresponds to the dosage cited in the Rote Liste® for the respective GnRH agonists for other indications of use for the subcutaneous or the intramuscular administration form, respectively. For intravenous administration of GnRH agonists the minimal daily dose is employed, cf. for example Klijn et al., 1982, *The Lancet*, 1213-1216.

The minimum treatment dose of the GnRH antagonists Cetrorelix®, Antrarelix®, Antide®, and Ramorelix® for subcutaneous and intramuscular administration forms in the above-cited list corresponds to the dosage described in the literature and used with other

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

|  |  |                                   |
|--|--|-----------------------------------|
| <b>STATEMENT CLAIMING SMALL ENTITY STATUS<br/>(37 CFR 1.9(f) &amp; 1.27(b))—INDEPENDENT INVENTOR</b> |  | Docket Number (Optional)<br>49477 |
|--|--|-----------------------------------|

Applicant, Patentee, or Identifier: VAN GROENINGHEN, Johannes Christianus

Application or Patent No.: PCT/DE98/01902

Filed or Issued: July 3, 1998  
Title: METHOD FOR RECOGNIZING AND DETERMINING GNRH RECEPTORS AND THE USE OF  
GNRH AGONISTS AND GNRH ANTAGONISTS AND OTHER GNRH RECEPTOR LIGANDS...

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- the specification filed herewith with title as listed above.
- the application identified above.
- the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- No such person, concern, or organization exists.
- Each such person, concern, or organization is listed below.

NAME: VAN GROENINGHEN, Johannes Christianus

ADDRESS: Ringofenstrasse 44, D-44287 Dortmund, Germany

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

**VAN GROENINGHEN**  
Johannes Christianus

NAME OF INVENTOR

  
Signature of inventor

Date

NAME OF INVENTOR

Signature of inventor

Date

NAME OF INVENTOR

Signature of inventor

Date

3/PPTS

514 Rec'd PCT/PTO 30 DEC 1993

METHOD FOR RECOGNIZING AND DETERMINING GNRH RECEPTORS AND  
THE USE OF GNRH AGONISTS AND GNRH ANTAGONISTS AND OTHER GNRH  
RECPETOR LIGANDS FOR THE TREATMENT WITH GNRH RECEPTORS OF  
TUMORS ORIGINATING IN THE BRAIN AND/OR NERVOUS SYSTEM AND/OR  
5 MENINGES AND/OR OF KAPOSI SARCOMA

The present invention relates to tumor diagnosis and therapy. In particular, it is directed to the diagnosis and therapy of tumors carrying GnRH receptors. The GnRH receptor is a well-known target in tumor therapy.

10 Post-operative treatment of prostate and mamma carcinomas with agonists of gonadotropin releasing hormone (GnRH, in the literature also referred to as luteinizing hormone releasing hormone; LH-RH) is a standard treatment; cf. Gonzalez-Barcena et al., 1994, *The Prostate* 24, 84-92; Emons and Schally, 1994, *Human Reproduction Update* 9, No. 7, 1364-1379.

15 Thus, in various steroid hormone (sexual hormone) dependent malignant tumors, such as mamma carcinoma, prostate carcinoma, ovarian carcinoma, and endometrial carcinoma, a double effect has been observed in clinical studies upon treatment with GnRH agonists:

- 1) an indirect anti-proliferative activity by uncoupling of the positive endocrine (estrogenous or androgenous) effect on tumor growth;
- 20 2) a direct anti-proliferative activity by an unknown mechanism via GnRH receptors in the tumor tissue itself; cf. Emons and Schally, 1994, *Human Reproduction Update* 9, 1364-1379.

25 The above-mentioned indirect effect due to steroid hormone dependence is known since decades for the prostate and the mamma carcinoma; cf. Gonzalez-Barcena et al., 1994, *The Prostate* 24, 84-92; Jonat et al., 1995, *European Journal of Cancer* 31A, 137-142.

30 The direct anti-proliferative effect of GnRH agonists and GnRH antagonists on e.g. prostate carcinomas, mamma carcinomas, and ovarian carcinomas has been confirmed by clinical studies. Some of the GnRH agonists employed in these treatments having a direct

anti-proliferative effect are known by the following trademarks of the medicaments approved in Germany: for example Zoladex®, Zoladex 10,8®, Zoladex Gyn®, Profact®-Depot, Profact pro injectione/nasal, Synarel®, Enantone Monats-Depot®, Uno-Enantone®, Enantone Gyn Monats-Depot®, Trenantone®, Suprecur®, Carcinil®, or 5 Decapeptyl® 0,5 mg/0,1 mg, Decapeptyl® Depot, Decapeptyl® Gyn as well as Decapeptyl® Diagnostik. An example for a GnRH antagonist examined in several studies is Cetrorelix® which is not yet approved as a medicament in Germany. Treatment with Cetrorelix® bears the disadvantage that no depot preparation exists which would be active e.g. for weeks. Other examples of GnRH antagonists used experimentally are Antarelix® 10 and Antide®, the latter also existing in one embodiment as an oral presentation form (Russel-Jones et al., 1995, Bioconjugate Chem. 6, 34-42).

Research with cell culture has revealed that GnRH receptors are present on human primary liver cell carcinomas and pancreas adenocarcinomas. In addition, the beginning of a biochemical metabolization with respect to cleavage of GnRH between tyrosine 5 and 15 glycine 6 in rat glioma and rat neuroblastoma has been described; cf. Tao et al., 1991, Neuropeptides 20, 125-131. Ligand binding of GnRH to the GnRH receptor and its signal transduction, however, take place in a different way, namely at the eighth amino acid of GnRH, arginine, and this exclusively occurs in the case of an intact conformation of the GnRH molecule and its amino acid side chains (Naor, Z., Schacham, Sh., Harris, D., Seger, 20 R., and Reiss, N., 1995, Signal Transduction of the Gonadotropin Releasing Hormone (GnRH) Receptor: Cross-Talk of Calcium, Protein Kinase C (PKC), and Arachnoidonic Acid. Cellular and Molecular Neurobiology, vol. 15, 527-545). In normal rat adenohypophysis where GnRH receptors reside, GnRH leads to an increased cAMP production, however, it is still unclear whether this is a direct or an indirect effect (paracrine 25 interaction). For the function of the GnRH receptor in rat including secretion of LH as well as an increased production of LH stimulated by GnRH, the biochemical metabolization of GnRH, e.g. by means of cAMP, plays only an indirect role (Abdilnour, G., and Bourne, G.A., 1995, Adenosine 3',5'-cyclic mono-phosphate and the self-priming effect of gonadotropin-releasing hormone. Molecular and Cellular Endocrinology, 107, 1-7). 30 Naturally, there were found GnRH receptors on human gonadotropin producing pituitary

adenomas (Alexander, J.P., and Klibanski, A., Gonadotropin-releasing Hormone Receptor mRNA Expression by Human Pituitary Tumors In Vitro, 1994, *Journal of Clinical Investigation*, 93, 2332-2339). To treat the indication Pubertas praecox e.g. due to the GnRH-producing hamartoma of the hypothalamus, GnRH agonists were also employed in 5 children in a symptomatic treatment of blocking gonadotropin-producing cells in the adenohypophysis (Mahachoklertwattana, P., Kaplan, S.L., Grumbach, M.M., The Luteinizing-Hormone-Releasing Hormone-Secreting Hypothalamic Hamartoma Is a Congenital Malformation: Natural History, 1993, *Journal of Clinical Endocrinology and Metabolism*, 77, 118-125).

10 In the case of glioma and other malignant tumors of ectodermal origin, such as malignant melanoma and in particular in the case of diffusely growing tumors in the nervous system or in the case of metastases (formation of disseminations, e.g. in other organs) life expectancy is not optimistic. The same is true for Kaposi sarcoma. Glioma refers to mainly brain-localized true tumors of the central nervous system (CNS) originating in the 15 neuroglia, i.e. from the covering and supporting tissue of the nervous system which is derived from ectoderm. These gliomas are present in various differentiation stages. Subtypes of glioma are spongioblastoma, oligodendrogloma, astrocytoma, glioblastoma, and retinoblastoma. In particular, the Glioblastoma multiforme (GBM) type of brain tumors is characterized by fast growth and extremely high recidivation rate (i.e. the percentage of 20 patients with brain tumor recurrence following surgical macroscopic excision).

25 Malignant melanoma occurring in the CNS, primary or as metastasis, as well as malignant melanoma which primarily occurs in the skin and/or malignant melanoma which disseminates (metastasizes) further in the skin and/or in other body organs belong to nerve system derived tumors; cf. Shamamian et al., 1994, *Cancer Immunol. Immunother.* 39, 73-83; Florenes et al., 1994, *Cancer Research*, 54, 354-356. Malignant melanomas are derived from neuroectoderm, an embryonic layer. Burg et al., 1997, *Deutsches Ärzteblatt* 94, 890-895, describe a tumor growth inhibiting effect of tamoxifen for the malignant melanoma. Furthermore, glioblastoma and malignant melanoma have several tumor markers in common; cf. Shamamian et al., 1994, *Cancer Immunol. Immunother.* 39, 73-83; Florenes et

al., 1994, Cancer Research 54, 354-356. In the case of metastases, the prognosis is very poor; cf. Burg et al., 1997, Deutsches Ärzteblatt 94, 890-895.

Tumors originating in brain and/or nervous system and/or the meninges further comprise the neuroblastoma and the medullablastoma which in their entirety have been  
5 classified as the so-called primitive neuroectodermal tumors, abbreviated as PNET. These tumors further include the pinealoma originating in pineal body parenchyma and/or primordial germ cells in the pineal body region or the brain median. Moreover, the pineal body is associated with the origin of craniopharyngioma (a tumor producing  $\beta$ -HCG or LH-like glycoprotein, respectively; cf. Tachibana et al., 1994, J. of Neurosurgery 80, 79-84)  
10 which is considered to be an ectodermal tumor and originates in the front/upper face of the pituitary.

Both for craniopharyngioma and meningioma which is considered to be a benign tumor originating in arachnoidal cover cells and often adhering firmly to the inner surface of the meninges (dura mater), progesterone receptors and estrogen receptors have been  
15 described. Furthermore, androgen receptors have also been established in the case of meningioma. In clinical studies using anti-progesterone medicaments, tumor-shrinking effects have been observed.

Up to now, the investigation of other therapies (different forms of chemotherapy, radiotherapie, etc.) in numerous clinical studies failed to provide a substantial improvement  
20 of the prognosis for tumors originating in brain and/or nervous system and/or the meninges. At the time being, the standard therapy in the case of Glioblastoma multiforme still consists of an as complete as possible surgical excision of the tumor followed by conventional radiotherapy. Under this standard therapy the statistically reported mean survival time is 9-13 months with individual variations and particularly a slightly better prognosis for younger  
25 patients having been observed.

About 30% of patients with recurrent Glioblastoma multiforme showed constant size or shrinking, respectively, of the inoperable residual brain tumor under sustained high-dosage of tamoxifen®, an anti-estrogen preparation. This tumor-inhibiting effect in glioblastoma treatment has not been attributed to its anti-estrogenic effect but to its  
30 inhibition of protein kinase C (an intracellular signal mediator); cf. Puchner et al.,

Zentralblatt für Neurochirurgie, Supplement 1996, 47. Jahrestagung Deutsche Gesellschaft für Neurochirurgie, page 44; Pollack et al., 1995, The Efficacy of Tamoxifen as an anti-proliferative Agent in vitro for Benign and Malignant Pediatric Glial Tumors, Pediatr. Neurosurgery 22, 281-288). Moreover, tamoxifen® is said to increase the sensitivity of 5 tumor cells for platinium-containing therapeutics as well as for radiotherapy.

For Glioblastoma multiforme (WHO grade IV astrocytoma) and for glioma with a lower grade of malignancy (WHO grade II-IV astrocytoma) steroid hormone receptors have been observed in a smaller percentage of the cases (cf. Paoletti et al., 1990, J. Neurosurgery, Characteristics and biological role of steroid hormone receptors in 10 neuroepithelial tumors, 73, 736-742). Up to now, an indirect anti-proliferative effect in the case of Glioblastoma multiforme and glioma grade II-IV has been observed in clinical studies in only about 30% of the cases by a response of the tumor to tamoxifen® (an anti-estrogen preparation)

Although recently, several relatively reasonable new developments in Glioblastoma 15 multiforme therapy have been described, the prognosis quod vitam for patients with Glioblastoma multiforme still remains poor because of the extremely high recurrence rate despite the therapy forms tried and tested so far and because of the lack of a specific therapy and early diagnostics.

On the one hand, the invention is based on the object of providing diagnostics which 20 detect tumors originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma already in an early stage, and on the other hand based on the object of providing a medicament for the therapy of such tumors which results in a better prognosis for all patients.

The invention relates to a method for the detection and determination of GnRH 25 receptors on malignant cells of a tumor originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma. The invention is further directed to providing a diagnostic kit for tumors originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma. Furthermore, the invention relates to the use of GnRH agonists and GnRH antagonists and of other ligands of GnRH receptors in the preparation of a

medicament for the treatment of tumors originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma.

The direct anti-proliferative effect of GnRH agonists on brain-derived tumors, e.g. Glioblastoma multiforme, has not been described to date. It has also been unknown that 5 GnRH receptors are present on human ectodermal tumors, such as Glioblastoma multiforme. Furthermore, it has been unknown up to now that GnRH receptors are present on Kaposi sarcoma.

The present invention contributes to the improvement in diagnosis and therapy of tumors originating in brain and/or nervous system and/or the meninges and/or of Kaposi 10 sarcoma by providing a suitable target for diagnosis and therapy.

The invention is further directed to the use of diagnostic kits for the detection of GnRH receptors in immunohistological diagnostics and/or for the detection of GnRH receptor mRNA for monitoring of the therapy, aftercare for early recurrence detection during follow-up of the residual tumor still present after operation, for example a low grade 15 glioma (G II-III WHO; cf. World Health Organization (WHO) classification of tumors of the central and peripheral nervous system, in: Kleihues et al., 1993, Histological Typing of Tumors of the Central Nervous System, Springer Verlag, Berlin-Heidelberg, New York-Tokyo) or for the detection of malignization in the sense of a Glioblastoma multiforme (G IV), and for early detection in risk groups for the screening for the presence of tumors, such 20 as Glioblastoma multiforme, originating in brain and/or nervous system and/or the meninges.

The kit according to the present invention may be used to detect GnRH receptors on cell membranes or in body liquids, such as blood, plasma, serum, urine or liquor, tissue extracts, tissue liquids, in vitro cell culture supernatants and cell lysates. The GnRH 25 receptor may for example be determined immunohistochemically on e.g. operatively excised tumor preparations or tissue cultures or, by means of a conventional radioimmuno assay, for example in body liquids. The diagnostic kit comprises a GnRH agonist and/or a GnRH antagonist and/or a monoclonal or polyclonal antibody against human GnRH receptors and/or one or more specific primers against GnRH receptors for example for the 30 amplification of the cDNA of a GnRH receptor in a reverse transcriptase-polymerase chain

reaction (RT-PCR). Detection of GnRH receptors is conducted in a manner known per se using well known immunological assays, in particular using enzyme-linked immunoabsorbent assays (ELISA), or in a particular embodiment using the methods described below for the detection and determination of GnRH receptors on degenerate cells.

5 In a preferred embodiment, the method of the present invention for the detection and determination of GnRH receptors on degenerate cells of a tumor originating in brain and/or nervous system and/or the meninges comprises the following steps of: a) homogenizing peroperatively obtained tumor tissue, b) separating the membrane fraction, c) determination of the protein concentration in the membrane fraction of b), d) determination of the 10 concentration of GnRH receptors in the membrane fraction of b). The present method is particularly useful for the detection and determination of GnRH receptors in tissue derived from Glioblastoma multiforme, medulloblastoma, pinealoma, neuroblastoma, craniopharyngioma, meningioma, chordoma, Ewing sarcoma, malignant melanoma, or Kaposi sarcoma. This method provides the possibility of tumor diagnosis.

15 In a particularly preferred embodiment, fresh human tumor tissue is collected for example during brain tumor surgery (peroperatively) followed by storage in liquid nitrogen. For GnRH receptor determination, the frozen tissue samples are ground and homogenized. In a centrifugation step, the samples are separated from larger tissue debris. The supernatant is again centrifuged. The resulting sediment (pellet) contains the membrane fraction which is 20 again homogenized to obtain an as homogenous membrane suspension as possible. The membrane suspension is used in the radio receptor assay for determination of GnRH receptors. First, the protein concentration in the membrane fraction prepared is determined photometrically in a conventional and known manner e.g. using the BioRad protein assay (BioRad, Munich). Determination of the GnRH receptor concentration is performed using a 25 known GnRH agonist, such as Buserelin® binding specifically to GnRH receptors in the membrane fraction prepared. Since the GnRH agonist has been radiolabeled, for example by <sup>125</sup>I, the concentration of bound radiolabeled GnRH agonist mirrors the concentration of GnRH receptors in the membrane fraction. The concentration of bound radiolabeled GnRH agonist is determined by means of radioactive counts per minute. Both low affinity/high

capacity and high affinity/low capacity GnRH receptor binding sites are evaluated (cf. Baumann, K., et al., 1993, Breast Cancer Research Treatment, vol. 25, page 37-46).

The invention further relates to the use of GnRH agonists or GnRH antagonists to prepare a medicament for the treatment of tumors originating in brain and/or nervous system and/or the meninges. In particular, the invention is directed to the use of GnRH agonists or GnRH antagonists to prepare a medicament for the treatment of Glioblastoma multiforme, medulloblastoma, pinealoma, neuroblastoma, craniopharyngeoma, meningioma, chordoma, Ewing sarcoma, malignant melanoma, or Kaposi sarcoma. GnRH receptors as well as a GnRH agonist/GnRH antagonist treatment have so far been described neither for craniopharyngeoma nor for meningioma or chordoma or Ewing sarcoma or malignant melanoma and also not for the Kaposi sarcoma. For these tumors, no blood-brain barrier exists, since they originally are extracerebral, intracranial or peripheral tumors. Therefore, the therapy according to the present invention using GnRH agonists and/or GnRH antagonists or conjugates thereof, respectively, is very advantageous. However, the blood-brain-barrier is permeable for GnRH since a two-direction-system, a bidirectional active transport of GnRH across the blood-brain-barrier exists (Barrera, C., Banks, W.A., Fasold, M.B., and Kastin, A.J., 1991, Effects of Various Reproductive Hormones on the Penetration of LHRH Across the Blood-Brain Barrier, Pharmacology, Biochemistry & Behaviour, vol. 41, 255-257). Thus the treatment by GnRH agonists/GnRH antagonists has advantages over the treatment with tamoxifen for which a blood-brain-barrier exists. For Ewing sarcoma and other peripheral forms of PNET outside of the nervous system, for malignant melanoma and for Kaposi sarcoma, the blood-brain-barrier generally does not play an essential role in the treatment with GnRH agonists/GnRH antagonists since these tumors in most of the cases arise and stay on the outside of the blood-brain-barrier.

**Table I**

List of GnRH agonists and GnRH antagonists which may be employed in the treatment of a tumor having GnRH receptors and originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma:

| <u>GnRH agonists:</u>           | <u>GnRH antagonists:</u>                               |
|---------------------------------|--|
| Pharmacological substance name  | Trade mark   |
| Leuprorelinacetate, Leuprorelin | Cetrorelix®, Asta Medica AG, Frankfurt/Main            |
| Triptorelinacetate, Triptorelin | Antarelix®, Asta Medica AG, Frankfurt/Main             |
| Buserelinacetate, Buserelin     | Antide®, Ares Saroni Int. SA, Lausanne,<br>Switzerland |
| Goserelinacetate, Goserelin     | Ramorelix®, Behringwerke, Marburg, Germany             |

5

Further examples of GnRH antagonists are:

- LHRH antagonists similar to Antide® as described in US patent 5,480,969  
(Bowers et al., date of patent: Jan. 2, 1996)
- LHRH peptide derivatives as described in the UK patent GB 2 246782 B  
10 (Albert, R., et al., patent published on 16/09/1992)
- LHRH antagonists as described in US patent 5,198,533  
(Schally et al., date of patent: Mar. 30, 1993)

15 The minimum treatment dose of the GnRH agonists in the above list corresponds to the dosage cited in the Rote Liste® for the respective GnRH agonists for other indications of use for the subcutaneous or the intramuscular administration form, respectively. For intravenous administration of GnRH agonists the minimal daily dose is employed, cf. for example Klijn et al., 1982, The Lancet, 1213-1216.

20 The minimum treatment dose of the GnRH antagonists Cetrorelix®, Antrarelix®, Antide®, and Ramorelix® for subcutaneous and intramuscular administration forms in the above-cited list corresponds to the dosage described in the literature and used with other

indications, cf. for example for Cetrorelix®: Gonzalez-Barcena et al., 1994, The Prostate 24, 84-92.

The minimum treatment dose of the GnRH agonists Cetrorelix®, Antrarelix®, Antide®, and Ramorelix® for the intravenous administration form in the above-cited list 5 corresponds to the dosage which is known for other indications at the proper approval board or described in the Deutsche Pharmazeutische Stoffliste or in the literature and is administered, for example for Antide®: Fattinger et al., 1996, Am. J. Physiol. 271 (Endocrinol. Metab. 34) E775-E787. The same is true for the GnRH antagonists as described in US patent 5,480,969, UK patent GB 2 246782 B, and US patent 5,198,533.

10 According to the invention, GnRH agonists and/or GnRH antagonists may be employed in any suitable form. For tumors within the blood-brain-barrier, direct injection, e.g. into the circulation, intraarterially directly into the nervous system circulation or intravenously, or injection in the liquor ways or local application in the tumor bed following surgery, directly after macroscopic tumor resection, peroperatively or with Ommaya® 15 reservoir, or another form of subcutaneous ventricular injection in the liquor ways is preferred. It is possible to use both GnRH agonists and GnRH antagonists because both bind as ligands to the GnRH receptor. Further, ligands which are specifically directed to the GnRH receptor may be used e.g. preferably human or humanized antibodies. In most cases it is preferable to ensure that the targeting agent primarily reaches tumor cells. Therefore 20 imaging methods using the ligand with tracers are a further aspect of the invention. If the ligand is localized mainly in the tumor, the ligand may be coupled to a cytotoxic agent, such as a radioisotope or another toxic substance such as ricin A or the like. Preferred GnRH agonists are cited in the Rote Liste which is explicitly incorporated herein by reference (Rote Liste, 1997, paragraph 50, part 3, pituitary hormones, 50038 to 50056, editor ROTE 25 LISTE® Service GmbH, Frankfurt/Main). Preferred GnRH antagonists which already have been clinically used in patients for other treatments are Cetrorelix® and Antarelix® from Asta Medica AG, Frankfurt/Main, Germany, and Antide® from Ares-Sarono Int. AG, Lausanne, Switzerland.

30 The above-mentioned GnRH agonists and GnRH antagonists may be administered in dosages approved for other treatments. There may also be used dosages established during

dose finding studies for the use of similar materials (substances, medicaments) such as somatostatin analogues in pituitary adenoma, glioblastoma or pancreas adenocarcinoma, or for phase II studies with GnRH analogues (agonists or antagonists) for other indications, e.g. mamma carcinoma, prostate carcinoma or ovarian carcinoma.

5 In a particular embodiment, the GnRH agonists or GnRH antagonists are conjugated with a gonadotropin or LH inhibitor, respectively, such as Gossypol® (cf. Flack et al., 1993, J. Endocrinol. Metab., Oral Gossypol in the Treatment of Metastatic Adrenal Cancer 76, 1019-1024; Poso, H., et al., The Lancet, 1980, 885) or with melatonin or a melatonin analogue (an agonist or antagonist) (cf. Lissoni et al., 1996, Increased Survival Time in  
10 10 Brain Glioblastomas by a Radioneuroendocrine Strategy with Radiotherapy plus Melatonin Compared to Radiotherapy Alone, Oncology 53, 43-46).

In the following an example for a preferred treatment protocol is described.

For the first time, the GnRH receptor concentration in cell membranes of human brain or nervous system tumor cells, i.e. the GnRH receptors on the membrane which are effective in vitro have been determined using a radio receptor assay. With the method according to the invention, the biological activity or specifically the active GnRH receptors, respectively, are determined. For this purpose, radiolabeled Buserelin®, a GnRH agonist, is used as a marker binding specifically to GnRH receptors. Based on radioactive counts of bound Buserelin® the GnRH receptor concentration may be determined. This detection has already been used for other tumors such as mamma carcinoma and the like. The method used according to the present invention measures the GnRH receptor concentration on cell membrane extracts of fresh human tumor tissue.

During peroperative resection of the tumor tissue on the one hand tissue is obtained and processed for pathological anatomical examination and on the other hand tumor tissue for GnRH receptor determination, e.g. in the manner described herein. Following pathological anatomical examination and confirmation of the histological diagnosis of a tumor originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma a prognosis may be made for a therapy success during treatment with GnRH agonists/GnRH antagonists with respect to the concentration of GnRH receptors present.

At a concentration of the GnRH receptor of more than 1000 amol/mg (= 1 fmol/mg) membrane protein the patient will be diagnosed as GnRH receptor-positive. Being not GnRH receptor-positive is no criterion for exclusion from treatment since no clinical exclusion criteria exist for GnRH agonist/GnRH antagonist treatment. The being GnRH receptor-positive of a patient is judged prognostically as a faster tendency of recidivation than that of being GnRH receptor-negative in the course of tumor growth under classical standard treatment wherein the GnRH receptor functions as a prognostic tumor marker. Also, being GnRH receptor-positive is considered to be particularly advantageous for the treatment with GnRH agonists/GnRH antagonists, and being GnRH receptor-positive or - negative provides a prognostic information of the therapy success to be expected so that GnRH receptor is a prognostic tumor marker in that treatment. The GnRH agonist/GnRH antagonist treatment is started immediately after pathological anatomical examination, e.g. postoperatively in the case of rapid section pathological diagnostics.

Following determination of the presence of GnRH receptors, a suitable ligand (GnRH agonist, GnRH antagonist or conjugates) is selected and administered to the patient from whom the tumor was derived, preferably after diagnostic imaging methods. Cf. MTT test literature: Hunter et al., 1993, *Europ. J. Surg. Oncology*, 242-249.

The treatment is continued as long as no complete remission has occurred. Criteria to judge the therapy effect are: A. tumor volume on MRT images and/or CAT scan images, B. recidivation-free survival, C. overall survival for initial application as well as D. Karnofsky and Spitzer indices. The dosage for administration which may be in any suitable form known to those skilled in the art is described above and below in this patent application.

The exact mechanism of action of GnRH agonists or GnRH antagonists on tumors is unknown. For the tumor types known so far having active GnRH receptors such as mamma carcinoma, prostate carcinoma and ovarian carcinoma, a locally regulatory autocrine-paracrine system has been proposed in the literature; cf. Irmer et al., 1995, *Cancer Research* 55, 817-822. For the tumors mentioned, anti-proliferative activities of GnRH agonists or GnRH antagonists have been described in the literature, both in vitro (Palyi et al., 1996, *Cancer Detection and Prevention*, 20, 146-152; Irmer et al., 1995, *Cancer Research*, 55,

817-822; Pati et al., 1995, Endocrinology, 136, 75-84) and in vivo or clinically, respectively; cf. Gonzalez-Barcena et al., 1994, The prostate 24, 84-92; Jonat et al., 1995, European J. of Cancer, 31A, 137-142; Emons and Schally, 1994, Human Reproduction Update 9, No. 7, 1364-1379; wherein this anti-proliferative activity goes beyond the anti-proliferative effect to be expected of reversible "chemical castration" by GnRH agonists.

For glioblastoma and glioma in a similar manner the following mechanism of action can be considered. In the literature (Constam et al., 1992, J. Immunology, 148, 1404-1410) the production of transforming growth factor  $\beta$  (TGF- $\beta$ ) by glioblastoma cells has been described. Growth factor TGF- $\beta$  has been described by Melcangi et al., 1995, Endocrinology, 136, 679-686, as a product of rat glia cells, i.e. normal non-tumor cells, which as a factor in vitro stimulates the natural GnRH production in hypothalamic cells. It has been postulated that GnRH produced and secreted locally by glioblastoma has a stimulating effect on the tumor growth which has also been known for TGF- $\beta$ . Also human glioblastoma cells and glioma cells, respectively, are able to secrete circulating immunosuppressive substances, mainly TGF- $\beta$ , and therefore may induce an adverse effect on cellular immune reactions. Besides a GnRH-stimulating function, the increase in TGF- $\beta$  presumably also has an immunosuppressive (defense inhibiting) effect on the cellular immunity of the patient due to which tumor growth is promoted and tumor size increases. For Glioblastoma multiforme, medulloblastoma, and malignant melanoma, this immunosuppressive phenomenon of TGF- $\beta$  has been described; cf. Stockhammer et al., 1995, J. of Neurosurgery 83, 672-681; Jennings et al., 1994, Hum. Pathol. 25, 464-475; Bzik et al., 1996, J. Cell Biochem. 62, 113-122; van Belle et al., 1996, Am. J. Pathol. 148, 1887-1894. This autocrine-paracrine growth regulating system may be reversed resulting in a decrease in tumor size. This reversion (also referred to as "negative feedback" in endocrinology) may be in principle effected by an excess of GnRH (competitive inhibition). This effect is even enhanced by using GnRH agonists or GnRH antagonists instead of GnRH. A result of this therapy is a decrease in TGF- $\beta$  production followed by a decrease in tumor size resulting therefrom. Also  $\beta$ -HCG plays an immunosuppressive role. According to the invention, also the LH- $\beta$  and  $\beta$ -HCG production, respectively, are inhibited by GnRH agonists or GnRH antagonists. Also, in GBM the EGF production is inhibited.

For the tumors originating in brain and/or nervous system and/or the meninges belonging to indication invention, reference is made to the World Health Organization (WHO) classification of tumors of the central nervous system which has been established in 1990 (Kleihues et al., 1993, Histological Typing of Tumors of the central nervous system, Springer Verlag, Berlin Heidelberg New York Tokyo). In addition to the tumors cited in the above-mentioned WHO classification, also malignant melanoma, Ewing sarcoma and the Kaposi sarcoma belong to the indication invention. Excluded from the indication invention are the pituitary adenoma, all metastases except Ewing sarcoma, melanoma and Kaposi sarcoma, lymphomas and hematopoietic tumors. Germ cell tumors such as chorion carcinoma are similar to malignant tumors of the placenta which are known for bearing GnRH receptors. Therefore, the germ cell tumors of the central nervous system belong to the present indication invention. The Kaposi sarcoma with multicentric occurrence in the body consists of cells of monoclonal origin (Rabkin et al., 1996, The New England Journal of medicine, 14, 988-993). It has specific antigens in common with skin neurofibroma, a tumor originating in the nervous system (Rudolph, P., et al., 1997, Am. J. Surg. Pathol. (US), 21(7), 791-800).

With respect to hormones, Kaposi sarcoma is similar to malignant placental tumors and meningioma since Kaposi sarcoma has  $\beta$ -HCG receptors as have these tumors and reacts anti-proliferatively to the administration of  $\beta$ -HCG as for example does the meningioma (Boyle-Wash et al., 1995, Effect of glycoprotein and protein hormones on human meningioma cell proliferation in vitro, Journal of Endocrinology, 145, 155-161; Albini et al., 1997, The beta-core Fragment of human chorionic gonadotropin inhibits growth of Kaposi sarcoma-derived cells and a new immortalized Kaposi sarcoma cell line, AIDS (US), 11(6), 713-721; Gill et al., 1996, The effects of preparations of human chorionic gonadotropin on aids-related Kaposi sarcoma, The New England Journal of Medicine, 335 (17), 1261-1269). Due to the analogy to meningioma, Kaposi sarcoma has GnRH receptors wherein the discovered autocrine connection of GnRH being known as the  $\beta$ -HCG releasing hormone in placenta and placental tumors plays a role (Lin et al., 1995, J. Clin. Endocrinol. Metab. 80, 580-585). The tumors cited above in the WHO classification of central nervous system tumors as well as malignant melanoma with  $\beta$ -HCG production

and/or  $\beta$ -HCG receptors carry GnRH receptors. The Ewing sarcoma belongs to the group of primitive neuroectodermal tumors (PNET) and is a peripheral form of these (Grier, H.E., 1997, The Ewing Family of Tumors. Ewing sarcoma and primitive neuroectodermal tumors. Pediatric Clin. North Am. (US), 44 (4), 991-1004).

5 The pineal gland (Glandula pinealis) is the origin of the production of the hormone melatonin which is a GnRH receptor expression stimulating hormone in metastasizing prostate carcinoma in the case of resistance during a GnRH agonist treatment (cf. Lissoni et al., 1997, European Urology 31, 178-181) and in addition has an anti-angiogenetic activity (Regelson, W., Pierpaoli, W., 1987, Cancer Invest., 5, 379-385). GnRH agonists and GnRH  
10 antagonists have an anti-mitotic and anti-proliferative activity, respectively, by inhibiting growth factors such as epidermal growth factor (Motta et al., 1996, J. Steroid Biochem. Molec. Biol., 56, 107-11, 1996). Epidermal growth factor is also present as a mitogen and, thus, as a positive growth factor, e.g. in Glioblastoma multiforme (Rao et al., 1996, Peptides (US), 17, 179-181). Thus, a melatonin-GnRH analogue conjugate reasonably  
15 combines an anti-mitotic and anti-angiogenetic activity on tumors such as glioblastoma and induces the further expression of GnRH receptors e.g. in Glioblastoma multiforme in order to avoid resistance against GnRH agonist/GnRH antagonist treatment by GnRH receptor depletion.

According to the present invention there are provided for the first time GnRH  
20 agonists or GnRH antagonists for the preparation of a medicament for the treatment of tumors originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma.

According to the invention, the GnRH agonists or GnRH antagonists as well as the conjugated GnRH agonists or GnRH antagonists are used to treat tumors originating in  
25 brain and/or nervous system and/or the meninges, for example Glioblastoma multiforme. The medicaments according to the present invention may be prepared in any manner known to the skilled artisan, in particular for subcutaneous, intramuscular, intravenous, intraspinal or subdural, respectively, or intranasal application or in the form of a sustained release implantation. The medicaments according to the present invention may also be administered  
30 via a subcutaneous ventricular cytostatic reservoir being connected to the ventricle wherein

the reservoir may be replenished by injections through the skin. The GnRH agonists may be administered in the same dosage as those which are for example used in the treatment of prostate, mamma carcinoma or endometriosis; cf. e.g. Rote Liste, 1997, paragraph 50, part 3, hypothalamic hormones, 50038 to 50056, Editor ROTE LISTE® Service GmbH, Frankfurt/Main, which is included herein explicitly by reference; cf. Annex A. The minimal dose corresponds to the dose cited in the Rote Liste for the respective GnRH agonists. For example, in the case of intraspinal or subcutaneous ventricular administration via a cytostatic reservoir the minimal dosage may be lower than that cited in the Rote Liste for the respective GnRH agonists. The maximal dose corresponds to the LD<sub>50</sub> value for the respective GnRH agonists. The dosage may be optionally increased or decreased following a finding of the GnRH receptor concentration obtained in a neurological manner. The frequency of application or daily dose, respectively, may also be found in the Rote Liste. Preferably, the medicaments are administered until complete remission (regression) of the tumor which may be evaluated neuroradiologically and clinically.

15 For subcutaneous administration, e.g. Carcinil®, Decapeptyl® 0,5 mg/0,1 mg or Uno-Enantone may be employed. As sustained release implantations for example Profact®-Depot, Zoladex®, or Enantone Monatsdepot may be administered. For intramuscular administration, e.g. Decapeptyl®-Depot, Decapeptyl®-Gyn, or Enantone-Gyn may be employed. For intranasal administration e.g. Profact®-Nasal, Suprecur®-Nasal, or 20 Synarel®-Nasal may be used. For intravenous administration or intranasal administration, respectively, for example Profact pro injectione/-nasal may be administered in the dosage given by Klijn, J.G., and De Jong, F.H. in Klijn, J.G., and De Jong, F.H., 1982, The Lancet, 1213-1216. The GnRH antagonists may be administered in a dosage which has been for example given for Cetrorelix® in Gonzalez-Barcena et al., 1994, The Prostate 24, 84-92, or 25 may be administered at minimum in the dosage as given for example for Antide® in Fattinger et al., 1996, Am. J. Physiol. 271 (Endocrinol. Metab. 34: E775-E787).

The following examples are intended to illustrate the invention and should not be construed as limiting the invention.

**Example 1: Determination of the concentration of GnRH receptors**

As an example for the determination of the concentration of GnRH receptors on cell membrane extracts of cell lines and/or cell cultures, the Decapeptyl® radio receptor assay is used with membranes (as described by Emons, G., et al., 1993, Cancer Research 53, 5439-5446). According to this protocol, the GnRH receptors are determined on a human cell line such as the human glioblastoma cell line U-87 MG or U-373MG (Pinski et al., 1994, Cancer Research 54, 5895-5901). In this test, the low affinity/high capacity as well as the high affinity/low capacity GnRH receptor binding sites are evaluated. Similar results as those described in Emons, G., et al., *supra*, for the cell lines EFO-21 and EFO-27 are obtained.

As another example for the determination of the concentration of GnRH receptors on cell membrane extracts of cell lines and/or cell cultures the LHRH radio receptor assay with labeled Triptorelin (Emons, G., et al., *supra*) is performed on a Kaposi sarcoma cell line such as the well known cell line KSY-1 or KS-SLK (Parkash et al., 1996, New England Journal of Medicine 335, 17, 1261-1269) and on a human malignant melanoma cell line such as the well known cell lines MV3 and BLM (Goldbrunner, R.H., et al., 1996, Anticancer Research 16 (6B), 3679-3687) obtaining similar results for the GnRH receptor determinations as described in Emons, G., et al., *supra*, for the cell lines EFO-21 and EFO-27.

**Example 2: Determination of the mRNA of GnRH receptors by means of RT-PCR**

As an example for the determination of GnRH receptor messenger RNA by means of RT-PCR for example RNA from the glioblastoma cell line U-87 MG or U-373MG is in a first reaction transcribed to cDNA. In a further reaction for example the 884 bp fragment of the pituitary GnRH receptor (Kakar, S., et al., Biochem. Biophys. Res. Comm., 1992, 289-295) or of the placental GnRH receptor (Leung, P.C.K., Biological Signals, 1996, 5, 63-69) or of the placental GnRH receptor gene (Lin, L., et al., J. Clinical Endocrinol. Metabolism, 1995, vol. 80, No. 2, 581-584) is amplified using specific primers in a reverse transcriptase polymerase chain reaction wherein the cDNA of a known GnRH receptor-positive cell line serves as the positive control. Then, the reaction products are visualized in a polyacrylamide (PAA) gel. On the PAA gel in lane 1 there may be seen the fragment length marker, in lane

2 a clear band of the 884 bp GnRH receptor PCR product in the MCF 7 positive control  
and also in the lane of the glioblastoma cell line a signal of an 884 bp product or other  
GnRH receptor splice variant (fragment) signals. This mRNA detection is performed similar  
to other GnRH receptor mRNA determinations, see for example Irmer et al., 1995, Cancer  
5 Research, 55, 817-822.

Example 3: Therapeutic in vitro study

Proliferation assay on cell cultures

A human cell line such as the well known human glioblastoma cell lines U-87MG or  
10 U-373MG (Pinski et al., *supra*) or a human cell line such as the well known Kaposi sarcoma  
cell lines KSY-1 or KS-SLK (Parkash et al., 1996, New England Journal of Medicine, 335,  
17, 1261-1269) or a human cell line such as the well known human malignant melanoma cell  
line MV3 or BLM (Goldbrunner, R.H., et al., 1996, Anticancer Research 16 (6B), 3679-87)  
or a human medulloblastoma cell line such as the well known cell line Daoy or D283 MED  
15 (Stockhammer et al., 1995, J. Neurosurgery, 83, 672-681) or human meningioma cell  
cultures (Boyle-Wash, E., et al., 1995, Journal of Endocrinology, 145, 155-161) are  
cultured as described by the above-mentioned authors for the above-mentioned cell lines  
and then treated as described by Emons, G., et al., 1993, *supra*, and Irmer, G., 1995, *supra*,  
with a concentration of the GnRH agonist Triptorelin, GnRH antagonist SB-75  
20 (Cetrorelix®) or GnRH antagonist Ramorelix® as described therein. Similar results to  
those described by Emons et al., Cancer Research, 53, 1993, 539-544, and Irmer, G., et al.,  
*supra*, were obtained.

Separately, the above-mentioned cell lines were also treated with an GnRH agonist,  
either with Goserelin (Zoladex®, Buserelin or Leuprorelin) or with a GnRH antagonist such  
25 as Antide® or Antarelix®. Similar anti-proliferative effects as those described by Pinski et  
al. or Irmer et al., *supra*, were observed.

Also separately, such cell lines were additionally treated each with one of the GnRH  
antagonists Cetrorelix®, Antarelix®, Antide®, and Ramorelix® or with one of the GnRH  
antagonists as described in US patent 5,480,969, US patent 5,198,533, or UK patent GB 2

246782 B wherein this treatment was performed similar to that reported in Emons et al., supra, for SB 75 (Cetrorelix®). A similar anti-proliferative effect occurs.

The cell lines cited above were also treated separately with monoclonal antibodies against a GnRH receptor antigen as described by Karande, A.A., et al., 1995, Mol. Cell.

5 Endocrinol. 114 (1-2), p. 51-56. A similar anti-proliferative effect is observed for the above cell lines as has been described by Ackermann, R.C., et al., 1994, Cancer Letters, 81, 177-184, for the OVCAR-3 cell line.

**Example 4: In vivo study in the model of xenotransplantation**

10 **An in vivo study with nude mice**

An effect of the treatment of tumor-implanted nude mice (Pinski et al., supra) each with one of the GnRH agonists Buserelin, Triptorelin, Goserelin, and Leuprorelin and each with one of the GnRH antagonists Cetrorelix® (SB-75), Antarelix®, Antide®, and Ramorelix® on the growth of malignant gliomas U-87 MG and U-373MG was proven by us using daily doses and controls in nude mice as have been described for the determination of the efficacy of similar peptides in Pinski et al., supra. Similar growth-inhibiting effects could be observed in the above tumors by treatment with the GnRH agonists and GnRH antagonists mentioned by us.

20 **Example 5: Phase I study**

Patients with non-resectable Glioblastoma multiforme in the condition after microsurgical resection and/or after external conventional radiotherapy and/or brachytherapy or patients with a diffusely, intraaxially growing brain tumor, multifocal tumor spreading or presence of a gliomatosis cerebri, respectively, a tumor volume of more than 65 ml or a minimal tumor diameter of more than 5 cm were treated with the GnRH agonist Buserelin administered intravenously as described by Klijn, J.G.M., et al., 1982, The Lancet, May 19, 12143-1214, and also as described therein by intranasal application as a permanent medication. As the effect of the treatment a reduction in tumor volume is observed on MRT or CT images, respectively. A recidivation-free survival longer than

described for the tamoxifen treatment method of glioma (Pollack et al., 1995, *Pediatr. Neurosurgery* 22, 281-288) has been observed.

**Example 6: Phase I study**

5        2 Patients with inoperable, stereotactically confirmed Glioblastoma multiforme after conventional radiotherapy were treated under permanent medication with Zoladex® in the dosage and administration form as cited for metastasizing mamma carcinoma in the Rote Liste. MRT controls reveal a significant reduction in tumor volume.

10      **Example 7: Phase II study**

Patients with histologically confirmed Glioblastoma multiforme after a first operation were treated (randomized controlled) with Zoladex® as described by Jonat et al., 1995, *European J. Cancer*, 137-142. Following radiotherapy, they are assigned to two groups. One group is treated with Zoladex® and one group without Zoladex® (or with Cetrorelix® and without Cetrorelix®, or with Antide® and without Antide®, or with Decapeptyl® or without Decapeptyl® etc.). The effects are similar to the metastasized perimenopausal mamma carcinoma. The percentage showing an actual significant therapy effect is evaluated according to the criteria of tumor volume, recidivation-free survival, overall survival following initial application and Karnofsky and Spitzer indices in a clinical neurological examination and under consideration of the other examination criteria (Sposto, R., et al., 1989, *J. Neurooncology*, 7, 165-177, and Kirby, S., et al., 1995, *J. Natl. Cancer Institute*, 87, 1884-1888, 1995). In MRT and/or CAT scan, a significantly higher reduction in tumor volume or significantly longer recidivation-free survival and significantly longer overall survival following initial application, respectively, than in the control group not treated with Zoladex® have been observed.

By using a method of gene therapy well-known to the skilled artisan retroviruses and antisense GnRH receptor vectors are stably transfected into glioma cells, and an anti-proliferative effect is observed.

Example 8: Collection of glioma tissue

During brain tumor operations (peroperatively) fresh human tumor tissue was collected dry in a small sterile dish without addition of medium and immediately transferred into a sterile standard plastic tube. The tube was sealed air-tight and after about 15 minutes 5 shock-frozen in a Dewar container (Union Carbide Cryogenic Equipment 35HC, ref. No. 103-139-T5) containing liquid nitrogen. The tissue samples were stored in liquid nitrogen for about 2 months until GnRH receptor determination.

Example 9: Tissue preparation

10 The frozen tissue samples were cleaned from residual blood and fat and cut into pieces of about 2 x 2 x 2 mm using a scalpel. The tissue samples were homogenized for 1 minute at maximum output in a Dismembrator II (B. Braun, Melsungen). The homogenized tissue was resuspended in 1000 µl of cold buffer 1 (10 mM tris-(hydroxymethyl)-aminomethane, pH 7.4, 4°C) and mixed as homogenous as possible. In a first centrifugation 15 step (800 x g, 10 minutes, 4°C) the sample was separated from larger tissue debris. The supernatant was again centrifuged (10.000 x g, 45 minutes, 4°C). The supernatant of the second centrifugation step was discarded, and the pellet containing the membrane fraction was resuspended in 1000 µl of cold buffer 1 and homogenized using a Polytron homogenizer three times for 4 seconds each to obtain an as homogenous membrane suspension as 20 possible. To this membrane fraction, 1000 µl of cold buffer 1 were added. This suspension was used in the determination of GnRH receptors in the radio receptor assay.

Example 10: Determination of the protein concentration

25 The BioRad reagent was diluted 1:5 with distilled water. 3.5 ml of this reagent were mixed with 50 µl of the membrane fraction prepared and incubated for 5 minutes. Photometric measurement of the protein concentration was carried out as a double determination at a lambda of 595 nm in a well-known manner. A human albumin protein standard which is correspondingly used for the measurement serves as the protein standard.

### Example 11: The radio receptor assay

The determination of the concentration of GnRH receptors was carried out in the membrane fraction of the tissue prepared as described above. The radio receptor assay comprised two different samples each of which is determined in fourfold: a) samples 5 containing the prepared membrane fraction, and b) control samples.

a) 300 µl buffer 2 (10 mM tris-(hydroxymethyl)-aminomethane, pH 7.4, 0.1% bovine serum albumin) and 100 µl of tracer (<sup>125</sup>I-Buserelin, 80.000 cpm/100µl) were added to 100 µl of membrane fraction.

b) For the controls, 250 µl buffer 2, 100 µl of tracer, 100 µl of membrane fraction and 10 50 µl GnRH analogue ( $10^{-5}$  M Buserelin) are mixed.

The individual samples were well mixed and then incubated for 90 minutes at 4°C. The radio receptor assay was stopped by addition of 500 µl of bovine gamma globulin solution (0.1% bovine gamma globulin, 0.15 M NaCl). Subsequently, 1000 µl of a 25% PEG-6000, 0.15 M NaCl solution were added.

15 The samples were again mixed until homogenous and incubated for 20 min at 4°C. Separation of the PEG-hormone receptor complexes was performed via a centrifugation step (1.600 x g, 30 minutes, 4°C) during which the complexes due to their higher mass form the pellet. The supernatant is removed carefully using a Pasteur pipette. The number of counts per minute serving as a basis for evaluation of the GnRH receptor content was then 20 determined in a Gamma counter (Berthold).

### Example 12: Examination of the radio receptor assay

Generally, several tissue samples were used in an experimental approach. To exclude a systematic error in the case of a negative result of all samples in one assay, a standard 25 sample from bovine pituitary tissue was examined in each of the assays in parallel to the tumor tissues. Thus, the detection of GnRH receptors in bovine pituitary tissues served as a positive control. The pituitary tissue was prepared similar to the tumor tissues and the membrane fraction was purified in a similar manner.

Example 13: Evaluation of the GnRH receptor content

The evaluation of the GnRH receptor content (fmol/mg of membrane protein) was carried out on the basis of the counts per minute (cpm), the specific binding, the amount of protein used, and the specific activity of the radiolabeled ligand.

5 The specific binding ( $B_{\text{spec}}$ ) is calculated from the difference of the mean value of the fourfold determination of total binding ( $B_0$ ) and the mean value of the fourfold determination of unspecific binding (NSB).

The amount of protein used is determined photometrically as described above under 3.

10 Data of the analogue  $^{125}\text{I}$ -Buserelin:

MG: 1253 g/mole

Specific Activity: 1470 mCi/mg

Activity of  $^{125}\text{I}$ -Buserelin solution 20  $\mu\text{Ci}/\text{ml}$

- 1470 mCi/mg  $^{125}\text{I}$ -Buserelin =  $54.4 \times 10^9 \text{ Bq}/\text{mg}$

15 - 1ml of  $^{125}\text{I}$ -Buserelin solution includes  $13.61 \times 10^{-9} \text{ g}$   $^{125}\text{I}$ -Buserelin with  $7.4 \times 10^6 \text{ Bq}$

-  $13.61 \times 10^{-9} \text{ g}/\text{ml}$   $^{125}\text{I}$ -Buserelin =  $10.9 \times 10^{-12} \text{ mole}$   $^{125}\text{I}$ -Buserelin,  $54.4 \times 10^9 \text{ Bq} = 44.4 \times 10^7 \text{ cpm}$ .

-  $10.90 \times 10^{-12} \text{ mole}$   $^{125}\text{I}$ -Buserelin =  $44.4 \times 10^7 \text{ cpm}$

- 1000 cpm correspond to  $0.247 \times 10^{-15} \text{ mole}$   $^{125}\text{I}$ -Buserelin.

20 For the calculation of the GnRH receptor concentration (fmol/mg of membrane protein) from the cpm values measured also the amount of protein used and the disintegration factor has to be considered. Thus, the equation for the calculation of the GnRH receptor content is the following:

25  $0.247 \times 10^{-15} \text{ mole}$   $^{125}\text{I}$ -Buserelin

$\frac{1000 \text{ cpm}}{\text{disintegration factor} \times \text{amount of protein}}$

**Table II**  
**Determination of the GnRH receptor concentration**

The results of the GnRH receptor determination using the radio receptor assay according to the invention of tissue samples of several patients are listed.

| Histological samples                     | ER<br>fmol/mg prot | PgR<br>Fmol/mg prot | GnRH rec.<br>atomol/mg prot | Finding           |
|--|--------------------|---------------------|-----------------------------|-------------------|
|  | 10                 | 20                  | 1000                        | negative          |
|  | 10-20              | 20-30               | 1000-3000                   | weakly positive   |
|  | 20                 | 30                  | 3000-5000                   | positive          |
|  | 50                 | 100                 | 5000                        | strongly positive |
| Chordoma                                 | 1                  | 1                   | 708                         |                   |
| GBM                                      | 1                  | 2                   | 2478                        |                   |
| GBM                                      | 1                  | 1                   | 895                         |                   |
| GBM                                      | 1                  | 1                   | 1111                        |                   |
| G II Glioma                              | 1                  | 1                   | 3635                        |                   |
| Meningeoma                               | 1                  | 74                  | 1                           |                   |
| Adenocarcinoma                           | 1                  | 1                   | 1                           |                   |
| GBM                                      | 1                  | 1                   | 7357                        |                   |
| Fibrillary                               |                    |                     |                             |                   |
| G II Astrocytoma                         | 1                  | 1                   | 1                           |                   |
| Meningeoma                               | 1                  | 177                 | 7444                        |                   |
| Meningeoma                               | 1                  | 550                 | 1588                        |                   |
| GBM                                      | 1                  | 1                   | 4466                        |                   |
| Additional values:                       |                    |                     |                             |                   |
| -Chordoma                                | 1                  | 1                   | 1117                        | weakly positive   |
| -Intraspinal<br>meningeoma               | 3                  | 7                   | 1640                        | weakly positive   |
| -Brain metastasis of<br>plate epithelium | 1                  | 1                   | 200                         | negative          |
| carcinoma of the<br>lung                 |                    |                     |                             |                   |
| -Normal brain tissue                     | 4                  | 1                   | 460                         | negative          |

5 ER=estrogen receptor, PgR=progesterone receptor, GnRH rec.=GnRH receptor

**Example 14: Proliferation assay using the human malignant melanoma cell line MV3**

The human melanoma cell line MV3 was cultured (in long-term culture in RPMI medium (Gibco Co.) with 1% Penstrep and 10% of heat-inactivated fetal calf serum). The proliferation assay was carried out with  $6 \times 10^2$  cells per well in 96 well plates. First, the 5 cells were removed from the culture flask with a 0.02 mM solution and then washed in standard PBS solution. Following centrifugation for 10 minutes (1200 g) the supernatant was discarded and the pellet resuspended in 1 ml medium. An aliquot of 20  $\mu$ l of the cells was diluted with trypan blue to obtain an 1:20 dilution. Trypan blue stains the necrotic cells. Then counting was performed in a Neubauer counting chamber. Evaluation was performed 10 by daily determination of 4 values starting at day 0 and multiplying the mean values of the cell counts  $\times 10^4$   $\times$  dilution factor 20 to obtain the cell count. During 5 days, the measurement was performed 4 x daily in a Biomec spectrophotometer.

The method for determination of tumor cell proliferation is described in Lü, H.Q., et al., 1996, Journal of Cancer Research and Clinical Oncology, 122, 335-342.

15 The cell line was treated with (Gly-OH10)-LHRH, the LHRH hormone (Figure 3) (Sigma Chemical Co., No. L8008) or Triptorelin, an LHRH agonist (Figure 2) (Sigma Chemical Co., No. L9761) or Antide, a LHRH antagonist (Figure 1) (Sigma Chemical Co., No. A8802).

20 In the concentrations of  $10^{-4}$  M,  $10^{-5}$  M, and  $10^{-6}$  M using medium as a negative control from day 4 on the following results were obtained:

25 Referring to Fig. 1: For Antide (GnRH antagonist) a clear inhibition of proliferation is seen in the high concentrations of  $10^{-4}$  M and  $10^{-5}$  M of 15% and 35%, respectively, (similar as described by Emons et al., 1993, *supra*, but with later onset as compared to the ovarian carcinoma cell lines used therein in which an anti-proliferative effect of the antagonists in one of the two cell lines occurred from day 1 on). At a concentration of  $10^{-6}$  M no inhibition of the proliferation was observed but a stimulation of the growth of 40%. This paradox in vitro effect of GnRH antagonists is similar to that described in Limonta et al., 1993, *J. Clin. Endocrinol. Metab.*, 76, 839-845, for prostate carcinomas with GnRH receptors. A similar in vitro effect for relatively low concentrations is also known for 30 tamoxifen in the MCF-7 mamma carcinoma cell line (Zänker, K., et al., 1995).

For Triptorelin (GnRH agonist) (see Figure 2) an inhibition of the proliferation of 15% was observed from day 4 on at the concentrations mentioned. In Emons et al., 1993, supra, this has been observed already starting from day 1 for both ovarian carcinoma cell lines under a Triptorelin treatment of  $10^{-5}$  M, and 40% inhibition was observed on day 6.

5 These findings indicate the presence of a direct anti-proliferative effect of Antide and Triptorelin on malignant melanoma. It has also been proven that GnRH receptors are present on the human malignant melanoma cell line MV 3 since binding of a non-ligand to the tumor cells can be excluded.

10 The graphs of Figures 1-3 prove that malignant melanoma MV3 is a LHRH homone-dependent tumor.

Thus, also in vitro the LHRH hormone functions as a positive growth factor. The function of LHRH hormone produced in an autocrine manner is inhibited by Antide and Triptorelin.

09/446996

5/4 Rec'd PCT/PTO 30 DEC 1999

CLAIMS

Art 234

1. Method for the detection and determination of GnRH receptors on tumor cells originating in brain and/or nervous system and/or the meninges and/or of Kaposi sarcoma comprising contacting said cells with a ligand for a GnRH receptor and determining if binding has occurred.
2. Method according to claim 1 characterized in that said ligand is an antibody.
- 10 3. Method according to claim 1 or 2 characterized in that said ligand is labeled.
4. Method according to claim 1 characterized in that the bound ligand is determined with labeled anti-ligand, preferably an antibody.
- 15 5. Method for the detection and determination of GnRH receptors on degenerate cells of a tumor originating in brain and/or nervous system and/or the meninges comprising:
  - a) homogenizing peroperatively collected tumor tissue;
  - b) separating the membrane fraction;
  - 20 c) determining the protein concentration in the membrane fraction of b); and
  - d) determining the concentration of GnRH receptors in the membrane fraction of b)to diagnose the above tumors.
- 25 6. Method according to claim 1 wherein the tissue is derived from a Glioblastoma multiforme, medulloblastoma, pinealoma, neuroblastoma, craniopharyngeoma, meningioma, chordoma, Ewing sarcoma, malignant melanoma, or Kaposi sarcoma.
7. Diagnostic kit for the practice of the method according to claims 1 to 6 comprising a  
30 ligand for a GnRH receptor.

8. Diagnostic kit according to claim 7 for the detection of GnRH receptors for immunohistological diagnostics, for monitoring of the therapy, aftercare, early recognition of recidivation, and early recognition of tumors originating in brain and/or nervous system and/or the meninges comprising either a GnRH agonist or a monoclonal or polyclonal antibody against GnRH receptors, or one or more specific primer for GnRH receptors, e.g. for the amplification of GnRH receptor DNA in a reverse transcriptase polymerase chain reaction (RT-PCR).

5

9. Diagnostic kit according to claim 3 comprising the use of the method according to claims 7 or 8.

10

10. Use of GnRH agonists or GnRH antagonists for the preparation of a medicament for the treatment of a tumor originating in brain and/or nervous system and/or the meninges and/or for the treatment of a Kaposi sarcoma.

15

11. Use of GnRH agonists or GnRH antagonists for the preparation of a medicament for the treatment of a Glioblastoma multiforme, medulloblastoma, pinealoma, neuroblastoma, craniopharyngeoma, meningioma, chordoma, Ewing sarcoma, malignant melanoma, or Kaposi sarcoma.

20

12. Conjugate of a GnRH agonist or GnRH antagonist to melatonin or to a melatonin analogue.

25

13. Use according to claim 10, whereby the GnRH agonists or GnRH antagonists are used in combination with a cytotoxic substance.

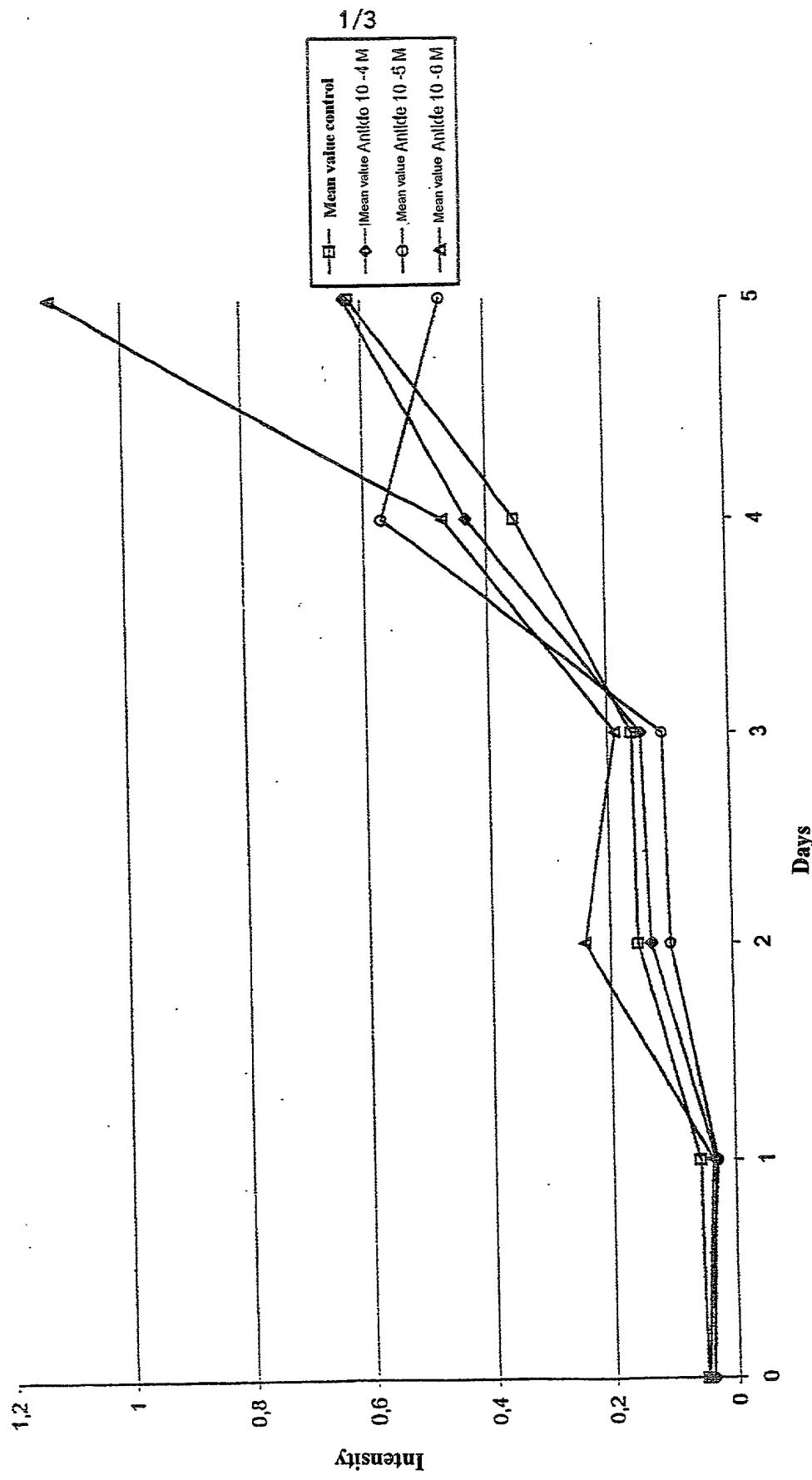
**A B S T R A C T**

5

The invention relates to a method for recognizing and determining GnRH receptors on abnormal cells of a tumour originating in the brain and/or nervous system and/or the meninges and/or on Kaposi sarcoma. The invention also relates to the preparation of 10 diagnostic kits for tumours originating in the brain and/or nervous system and/or the meninges and/or for Kaposi sarcoma. The invention further relates to the use of GnRH agonists and GnRH antagonists and/or other ligands for GnRH receptors for producing medicinal drugs for the treatment of tumours originating in the brain and/or nervous system and/or the meninges and/or for the treatment of Kaposi sarcoma.

09/446996

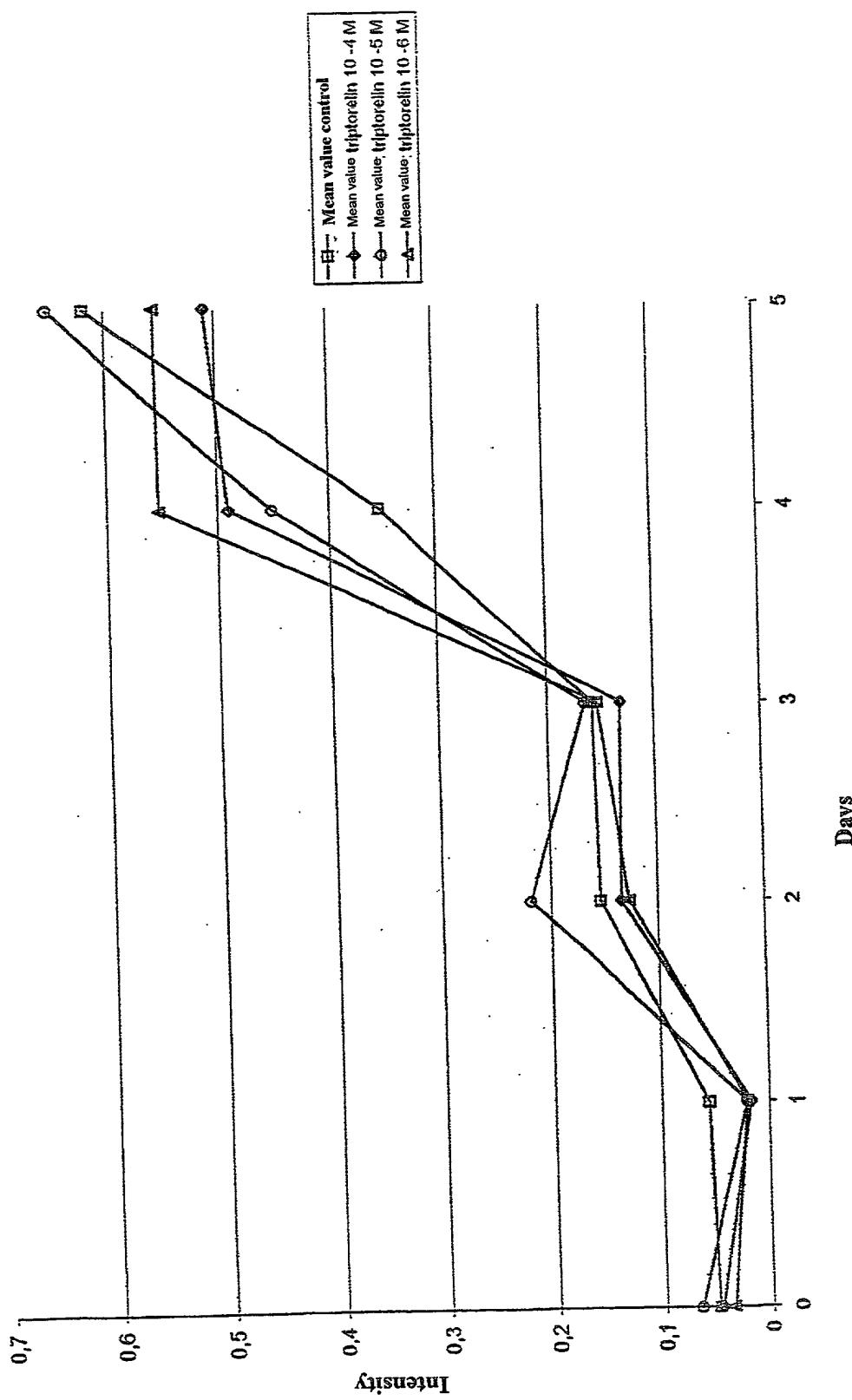
Figure 1 Antide



09/446996

2/3

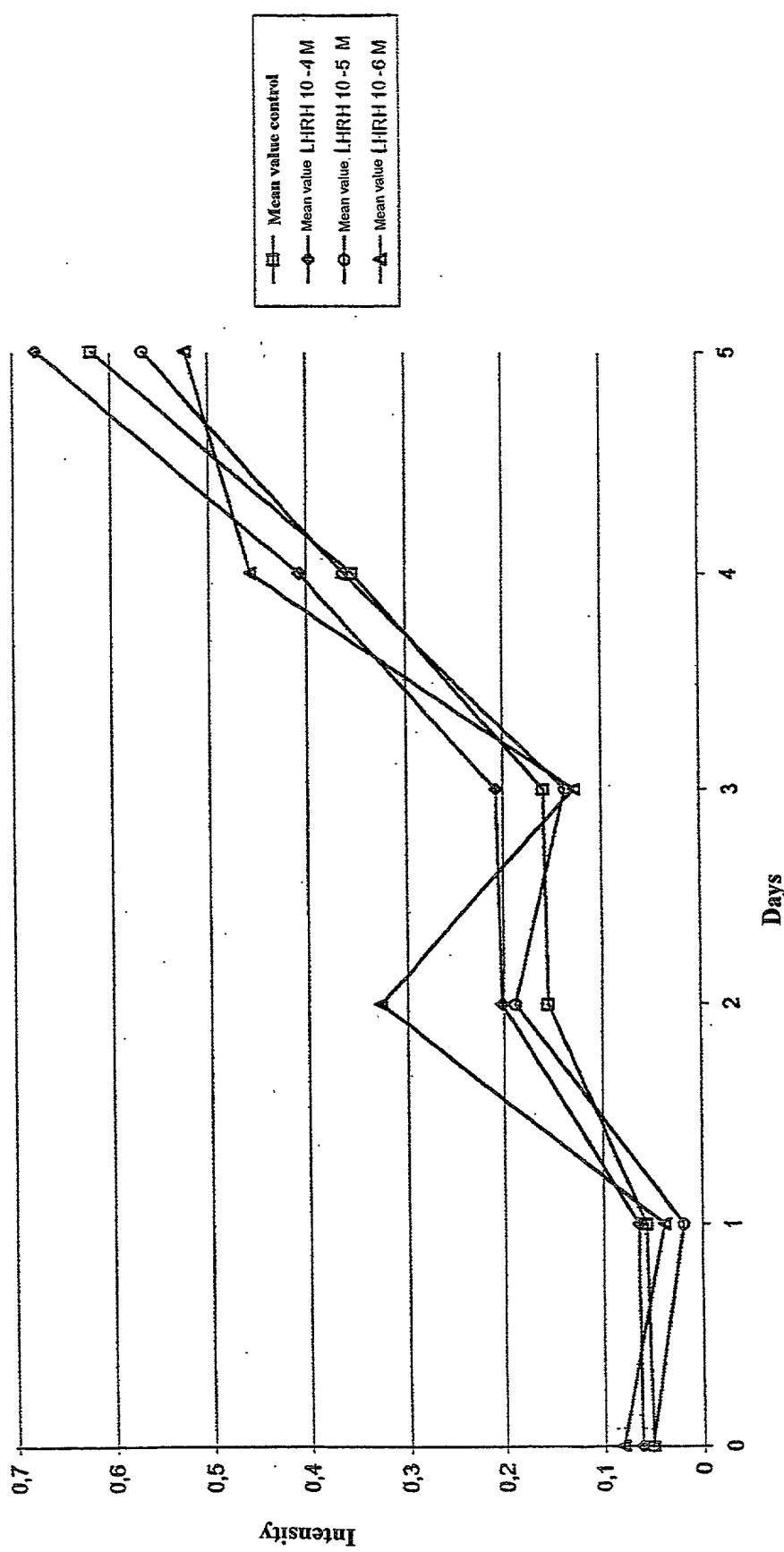
Figure 2 triptorelin



09/446996

3/3

Figure 3 LHRH Hormone



Please type a plus sign (+) inside this box →

PTO/SB/01 (12-97)

Approved for use through 9/30/00. OMB 0651-0032

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

**DECLARATION FOR UTILITY OR  
DESIGN  
PATENT APPLICATION  
(37 CFR 1.63)**

Declaration Submitted with Initial Filing      OR       Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

|                          |                        |
|--------------------------|------------------------|
| Attorney Docket Number   | 49477                  |
| First Named Inventor     | VAN GROENINGHEN, J. C. |
| <b>COMPLETE IF KNOWN</b> |                        |
| Application Number       | /                      |
| Filing Date              |                        |
| Group Art Unit           |                        |
| Examiner Name            |                        |

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**METHOD FOR RECOGNIZING AND DETERMINING GNRH RECEPTORS AND THE USE OF GNRH AGONISTS AND GNRH ANTAGONISTS AND OTHER GNRH . . .**

the specification of which *(Title of the Invention)*

is attached hereto  
OR

was filed on (MM/DD/YYYY)  as United States Application Number or PCT International

Application Number  and was amended on (MM/DD/YYYY)  (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application Number(s) | Country | Foreign Filing Date (MM/DD/YYYY) | Priority Not Claimed   | Certified Copy Attached?   |
|-------------------------------------|---------|----------------------------------|--|--|
|                                     |         |                                  | YES  | NO   |
| 197 28 737.9                        | DE      | 07/04/1997                       | <input type="checkbox"/><br><input type="checkbox"/><br><input type="checkbox"/><br><input type="checkbox"/> | <input type="checkbox"/><br><input type="checkbox"/><br><input type="checkbox"/><br><input type="checkbox"/> |

Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

| Application Number(s) | Filing Date (MM/DD/YYYY) |  |
|-----------------------|--------------------------|--|
|                       |                          | <input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto. |

[Page 1 of 2]

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Inventor(s): VAN GROENINGHEN, Johannes Christianus

Title: METHOD FOR RECOGNIZING AND DETERMINING GNRH RECEPTORS AND THE USE OF GNRH AGONISTS AND GNRH ANTAGONISTS AND OTHER GNRH RECEPTOR LIGANDS FOR THE TREATMENT WITH GNRH RECEPTORS OF TUMOURS ORIGINATING IN THE BRAIN AND/OR NERVOUS SYSTEM AND/OR MENINGES AND/OR OF KAPOSI SARCOMA

## POWER OF ATTORNEY

The specification of the above-identified patent application

is attached hereto  
 was filed on 3 July 1998 as application Serial No. Nationalized from PCT/DE98/01902

I hereby revoke all previously granted powers of attorney in the above-identified patent application and appoint the following attorneys to prosecute said patent application and to transact all business in the Patent and Trademark Office connected therewith:

1. Peter F. Corless (Reg. No. 33,860)

Please address all correspondence and telephone calls to Peter F. Corless in care of:

DIKE, BRONSTEIN, ROBERTS & CUSHMAN, LLP  
130 Water Street  
Boston, Massachusetts 02109-4280  
UNITED STATES OF AMERICA

The undersigned hereby authorizes the U.S. attorneys named herein to accept and follow instructions from Dr. Volker Vossius Patentanwaltskanzlei · Rechtsanwaltskanzlei as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the undersigned.

  
Inventor: VAN GROENINGHEN, Johannes Christianus

Date: 7-12-1999

Please type a plus sign (+) in the box below.

Approved for use through 9/30/00. OMB 0651-0032

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

PTO/SB/01 (12-97)

## DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

| U.S. Parent Application or PCT Parent Number | Parent Filing Date (MM/DD/YYYY) | Parent Patent Number (If applicable) |
|--|---------------------------------|--------------------------------------|
|  |                                 |                                      |

Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:  Customer Number  →  Place Customer Number Bar Code Label here  
**OR**  
 Registered practitioner(s) name/registration number listed below

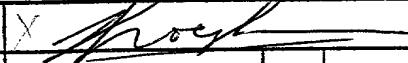
| Name | Registration Number | Name | Registration Number |
|------|---------------------|------|---------------------|
|      |                     |      |                     |

Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to:  Customer Number or Bar Code Label  OR  Correspondence address below

|         |           |     |  |
|---------|-----------|-----|--|
| Name    |           |     |  |
| Address |           |     |  |
| Address |           |     |  |
| City    | State     | ZIP |  |
| Country | Telephone | Fax |  |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

|                                      |   |                        |         |              |             |         |
|--------------------------------------|---|------------------------|---------|--------------|-------------|---------|
| Name of Sole or First Inventor:      | <input type="checkbox"/> A petition has been filed for this unsigned inventor       |                        |         |              |             |         |
| Given Name (first and middle if any) |   | Family Name or Surname |         |              |             |         |
| Johannes Christianus                 |   | VAN GROENINGHEN        |         |              |             |         |
| Inventor's Signature                 |  |                        |         | Date         |             |         |
| Residence: City                      | Dortmund  | State                  | Country | Germany (DE) | Citizenship | NL      |
| Post Office Address                  | Ringofenstrasse 44, D-44287 Dortmund, Germany                                       |                        |         |              |             |         |
| Post Office Address                  |   |                        |         |              |             |         |
| City                                 | Dortmund  | State                  | ZIP     | 44287        | Country     | Germany |

Additional inventors are being named on the \_\_\_\_\_ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto